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Research report

Is unpredictable chronic mild stress (UCMS) a reliable model to study depression-induced neuroinflammation?

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ABSTRACT

Unipolar depression is one of the leading causes of disability. The pathophysiology of depression is poorly understood. Evidence suggests that inflammation is associated with depression. For instance, pro-inflammatory cytokines are found to be elevated in the peripheral blood of depressed subjects. Cytokine immunotherapy itself is known to induce depressive symptoms. While the epidemiological and biochemical relationship between inflammation and depression is strong, little is known about the possible existence of neuroinflammation in depression. The use of animal models of depression such as the Unpredictable Chronic Mild Stress (UCMS) has already contributed to the elucidation of the pathophysiological mechanisms of depression such as decreased neurogenesis and HPA axis alterations. We used this model to explore the association of depressive-like behavior in mice with changes in peripheral pro-inflammatory cytokines IL-1β, TNFα and IL-6 level as well as the neuroinflammation by quantifying CD11b expression in brain areas known to be involved in the pathophysiology of depression. These areas include the cerebral cortex, the nucleus accumbens, the bed nucleus of the stria terminalis, the caudate putamen, the amygdala and the hippocampus. The results indicate that microglial activation is significantly increased in the infralimbic, cingulate and medial orbital cortices, nucleus accumbens, caudate putamen, amygdala and hippocampus of the mouse brain as a function of UCMS, while levels of pro-inflammatory cytokines did not differ among the groups. This finding suggests that neuroinflammation occurs in depression and may be implicated in the subject's behavioral response. They also suggest that UCMS could be a potentially reliable model to study depression-induced neuroinflammation.

1. Introduction

Unipolar depression is the leading cause of disease burden in high-income countries [1]. Initial treatment with conventional antidepressants fails to improve almost one-third of the patients [2] due in part to an incomplete understanding of pathophysiology of the disease. Clinical and preclinical data suggest that depression is associated with the activation of the immune system, which manifests as inflammation. This finding has complicated the pathophysiological picture of the disease [3]. In particular, episodes of depression have been characterized by an increase in the levels of various pro-inflammatory cytokines such as tumor necrosis factor (TNFα) and interleukin-6 which have been concluded to be raised in depressed subjects in a meta-analysis for instance [4]. When challenged with a social stress, mice sustain an increase in cytokines and corticosterone in peripheral blood [5]. Depression-like behavior can also be induced by the peripheral administration of cytokines and alleviated by their antagonists [6] as well as by antidepressant treatment which promote anti-inflammatory cytokine IL-10 [7], for a review, see [8]. Pretreatment with a selective serotonin reuptake inhibitor (SSRI) can also reduce the incidence of depression in patients undergoing interferon immunotherapy [9].

Microglia are brain equivalent of peripheral immune cells i.e., lymphocytes. They are found throughout the brain and constitute the prime group of cells which are activated in response to immune challenge [9,10]. Microglia's activation alters the subject's response to stress. It is beneficial in the beginning, as for long term potentiation and neurogenesis in hippocampus, mediated through

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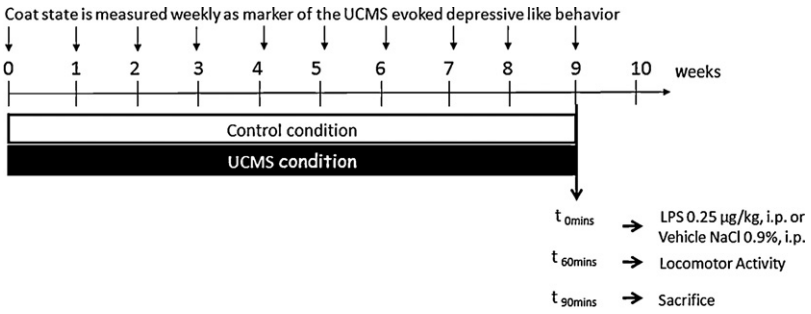

Fig. 1. The experimental protocol showing the weeks during which mice were exposed to UCMS. After 9 weeks of the UCMS or control condition, mice were injected with either LPS (0.25 μg/kg) or NaCl (0.9%) and tested for locomotor activity before being sacrificed. The brains were then collected for immunohistochemistry.

neurotransmitters and inflammatory cytokines like glucocorticoids and IL-1 [11–13]. If, however, it continues for long, it can progress towards neuronal injury and degeneration [11,14]. Any type of stress such as traumatic brain injury [15], cerebrovascular accidents [16], neurodegenerative diseases [17] and infections [18] can lead to microglial activation. Moreover, microglial activation and the resulting neuroinflammation may be implicated in the pathophysiology of neurodegenerative disorders and depressive illness. Increasing epidemiological data suggest a relationship among inflammation, depression and neurodegeneration. Epidemiological studies have shown that depressed subjects are more likely to develop degenerative diseases such as Alzheimer's disease (AD) [19] or Parkinson disease (PD) in older ages [20]. Further preclinical studies suggest that neuroinflammation could be one of the mechanisms implicated in this association. Norepinephrine (levels of which are thought to be decreased in depression) has been shown to suppress Aβ-induced cytokine and chemokine production and to increase microglial migration and the phagocytosis of Aβ [21], while antidepressants such as imipramine [22] have been shown to limit amyloid brain deposition in the mouse. This latter effect is mediated by a decrease in TNF-α expression. Brain imaging studies in humans have shown that depression and/or depressive-like states are associated with morphological (e.g., decreased volume of the hippocampus and increased amygdala volume) as well as functional/molecular brain alterations (such as decreased activation of the temporal cortex and insula; increased activity in the cerebellum, ventromedial prefrontal and anterior cingulate cortices; increased activation of the amygdala; decreased hippocampal neurogenesis; and altered BDNF levels in the nucleus accumbens) [23]. It can therefore be hypothesized that some of these brain changes could be related to neuroinflammatory process and particularly microglial activation. However, at this time, very few studies have attempted to examine the effect of stress-induced microglial activation in the various brain areas known to be implicated in the pathophysiology of depression. Consequently, the objective of this study was to assess the ability of the unpredictable chronic mild stress (UCMS) model, a validated rodent model of depression, to elucidate the role of neuroinflammation in the pathophysiology of depression and any related increase in the risk of neurodegenerative disorders. We therefore sought to measure microglial activation in mice exposed to the UCMS procedure in a subset of brain regions, namely, the cortex (infralimbic, prelimbic, medial orbital, cingulate), nucleus accumbens (core, shell), caudate putamen, amygdala, bed nucleus of the stria terminalis and hippocampus (Cornu Ammonis 1 & 3, dentate gyrus, polymorphous layer and molecular layer of the dentate gyrus). We also compared these results to the effects of bacterial lipopolysaccharide, a well-known activator of microglia. Furthermore, to compare the stress-induced neuroinflammation with the peripheral immune alterations, we measured serum levels of pro-inflammatory cytokines.

2. Material and method

2.1. Animals

Two groups of 7-week-old male BALB/cByJ@Rj mice (Centre d'élevage JANVIER Le Genest-St-Isle France) were subjected to unpredictable chronic mild stress (UCMS) ($n = 14$) or kept in standard housing conditions as controls ($n = 14$) for 9 weeks. Control mice were housed in groups of five, while UCMS mice were housed individually. The light and dark cycle was reversed (i.e., lights on from 8 p.m. to 8 a.m.). Room temperature was maintained at 22 °C ± 2 °C. Food and water were provided ad libitum. All procedures were carried out in the dark phase of the cycle and in accordance with the veterinary service (agreement number C37-261-2), the Ethics Committee for Animal Experimentation (Val de Loire n°2011-06-10), the European Community Council directive 86/609/EEC and the Ministry of Agriculture of France. A general scheme of the experimental protocol is presented in Fig. 1.

2.2. Unpredictable chronic mild stress (UCMS)

It is a variation of the chronic stress procedure described in rats by Willner et al. as a naturalistic rodent model of depression [24]. This protocol consisted of the chronic exposure of mice to various randomly scheduled, low-intensity social and environmental stressors (e.g., social stress, wet bedding, frequent sawdust changes, predator sounds, restraint stress, alterations of the light and dark cycle, tilting of the cages at 45° and the addition of rat droppings to mouse cages). The application of the different stressors was randomized each week to maximize the degree of unpredictability. For ethical reasons, the stress procedure did not involve food or water deprivation. Coat state was measured weekly as one of the markers of UCMS-induced depressive-like behavior [25,26]. The total score given for coat state was the sum of the scores obtained from seven body parts. Two independent, blinded observers performed evaluation of the coat state. After testing inter-observer reliability, the means of the results reported by both observers were statistically analyzed. This index has been pharmacologically validated in previous studies using BALB/c mice. Mice were sacrificed after 9 weeks of exposure to UCMS. The current protocol did not include any antidepressant treatment groups as it was intended to investigate neuroinflammation only.

2.3. Treatment with bacterial antigen lipopolysaccharide (LPS)

Ninety minutes before sacrifice, mice were intraperitoneally injected with the bacterial antigen lipopolysaccharide (LPS) (Sigma–Aldrich MO 63103 USA) at 0.25 μg/kg body weight prepared in 0.9% normal saline at a volume of 10 ml/kg body weight (UCMS $n = 7$ and control $n = 6$). Fifteen mice were injected with normal saline at 10 ml/kg body weight (UCMS $n = 7$ and control $n = 8$). The behavioral effects of LPS were measured by recording locomotor activity one hour after the injection. Locomotor activity was measured for 30 min using an actimeter, which assessed the activity of mice in their home cages. The cage was placed in the center of the device, which consisted of a plane crossed by photo-beam detectors. Movement of the animal was automatically detected and then scored. Higher scores reflected more mouse movement. Transparent cages with 1/3rd of the normal sawdust level were used for this purpose so as to minimize interference with activity detection.

2.3.1. Sacrifice

After the locomotor activity scores were recorded, mice were anesthetized with pentobarbital (injected intraperitoneally at a dose of 40 mg/kg body weight in a volume of 10 ml/kg body weight). Peripheral blood was collected and subjected to centrifugation for 15 min at 5000 rpm. Serum was collected and frozen at −80 °C until the levels of pro-inflammatory cytokines could be measured (Microdosages par la technologie xMAP luminex Plate-forme phénotypage du petit animal et microdosages. Hôpital Saint-Antoine–Bâtiment Kourirsky Paris France). The transcardiac perfusion of mice with 180 ml of 4% paraformaldehyde (PFA) followed. Brains were dissected out, post-fixed in PFA for 2 h and then cryoprotected in 20%